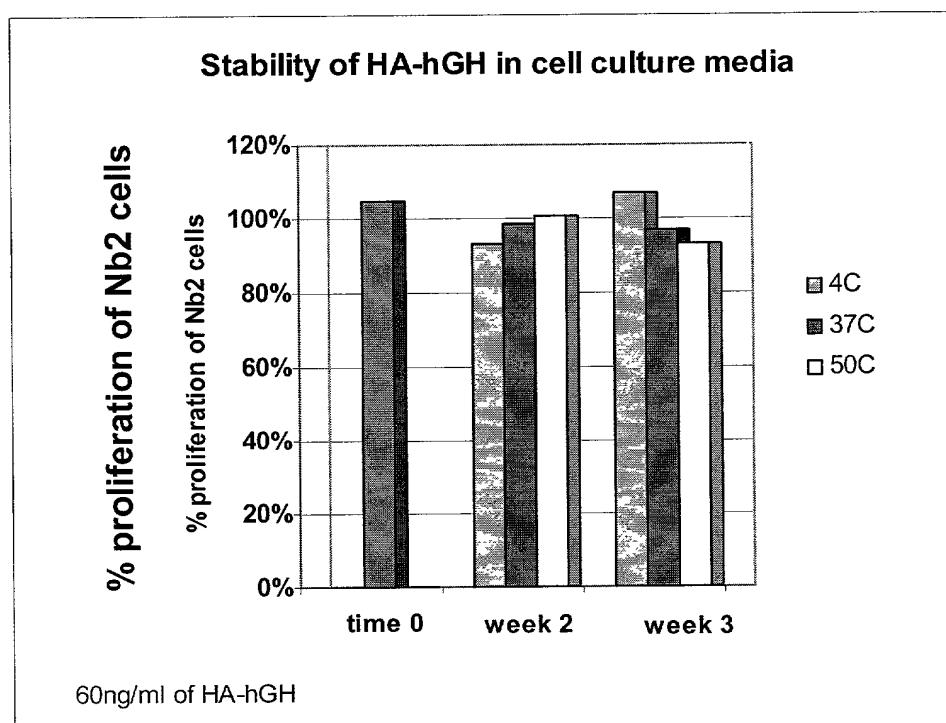
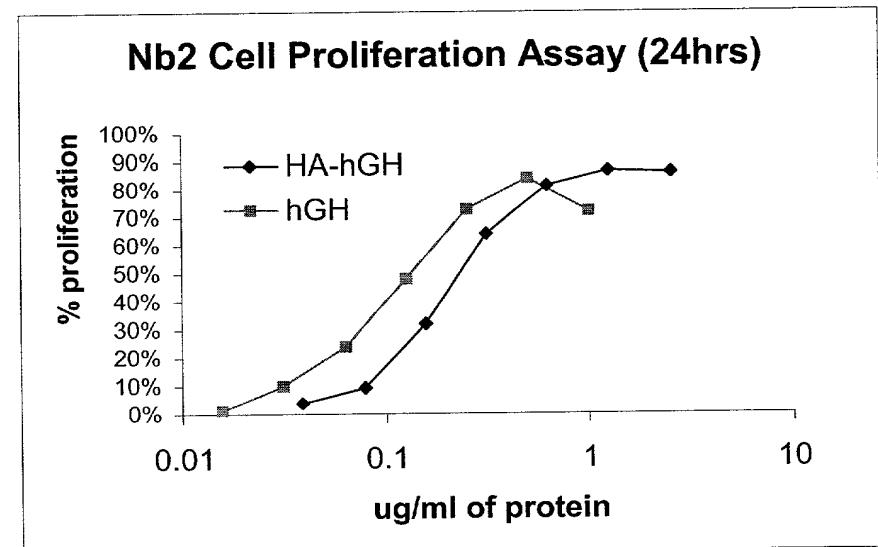


**Figure 1**

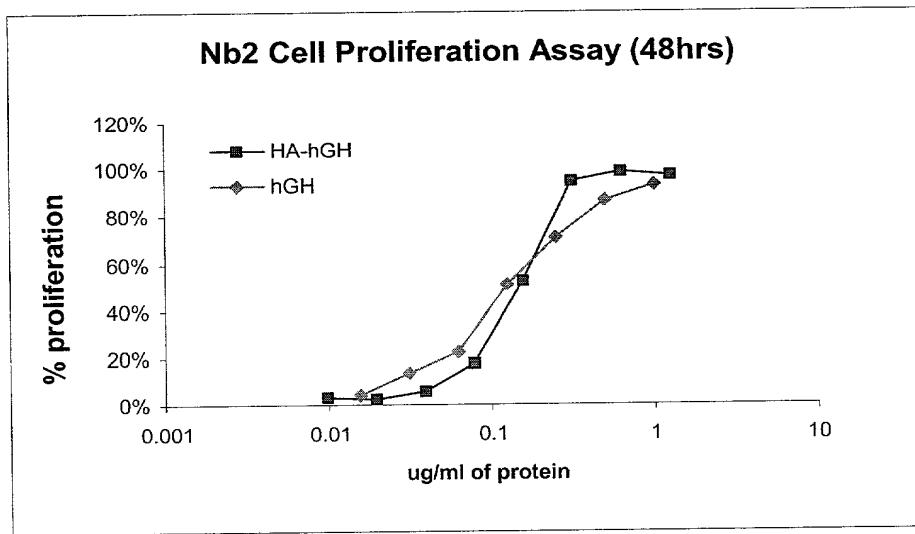


**Figure 2**

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**Figure 3A**



**Figure 3B**

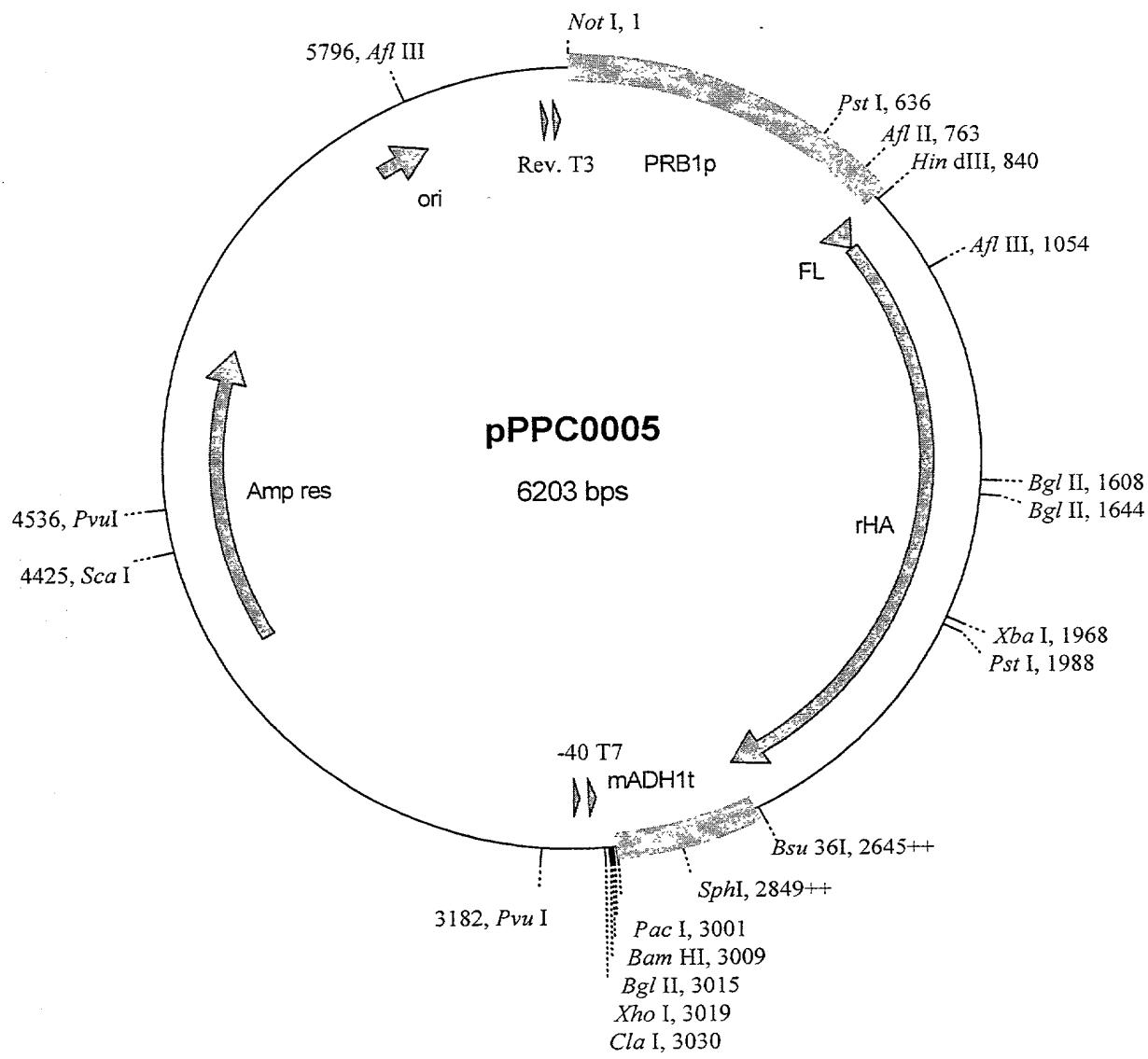


Figure 4

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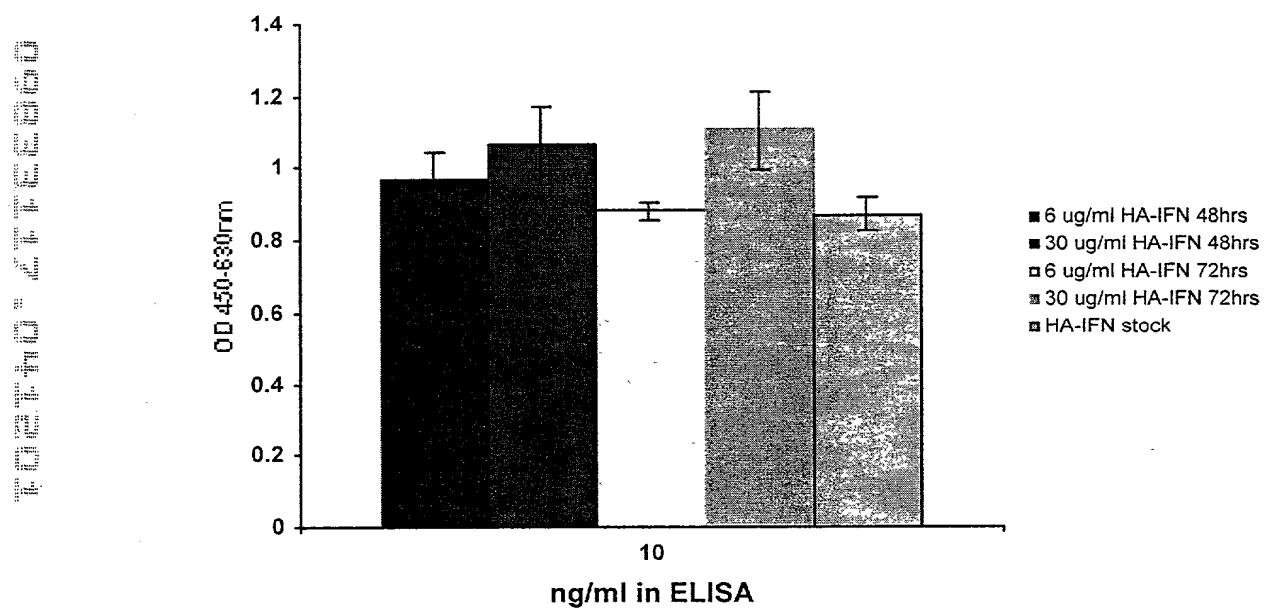


Figure 5

Figure 6

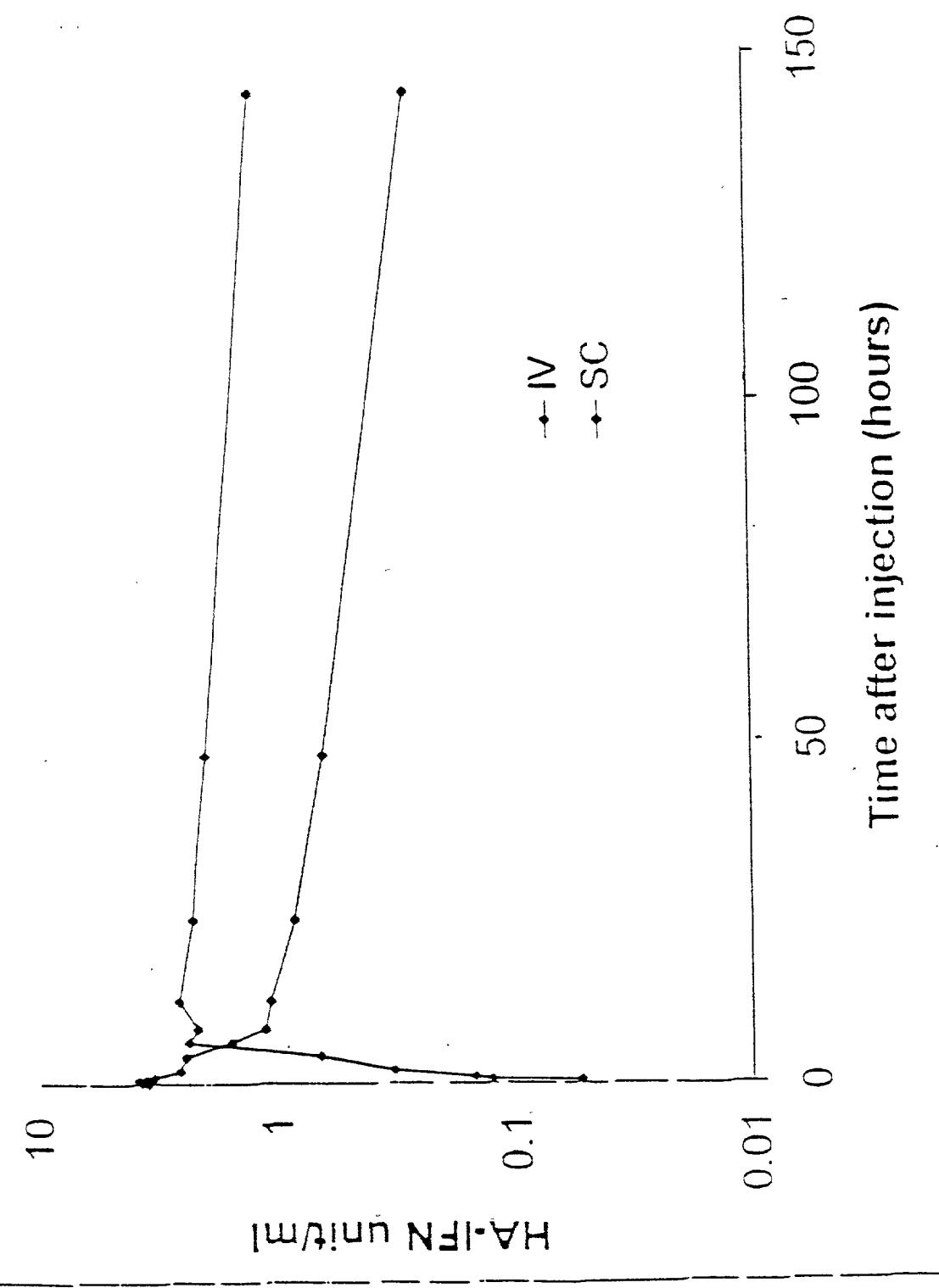
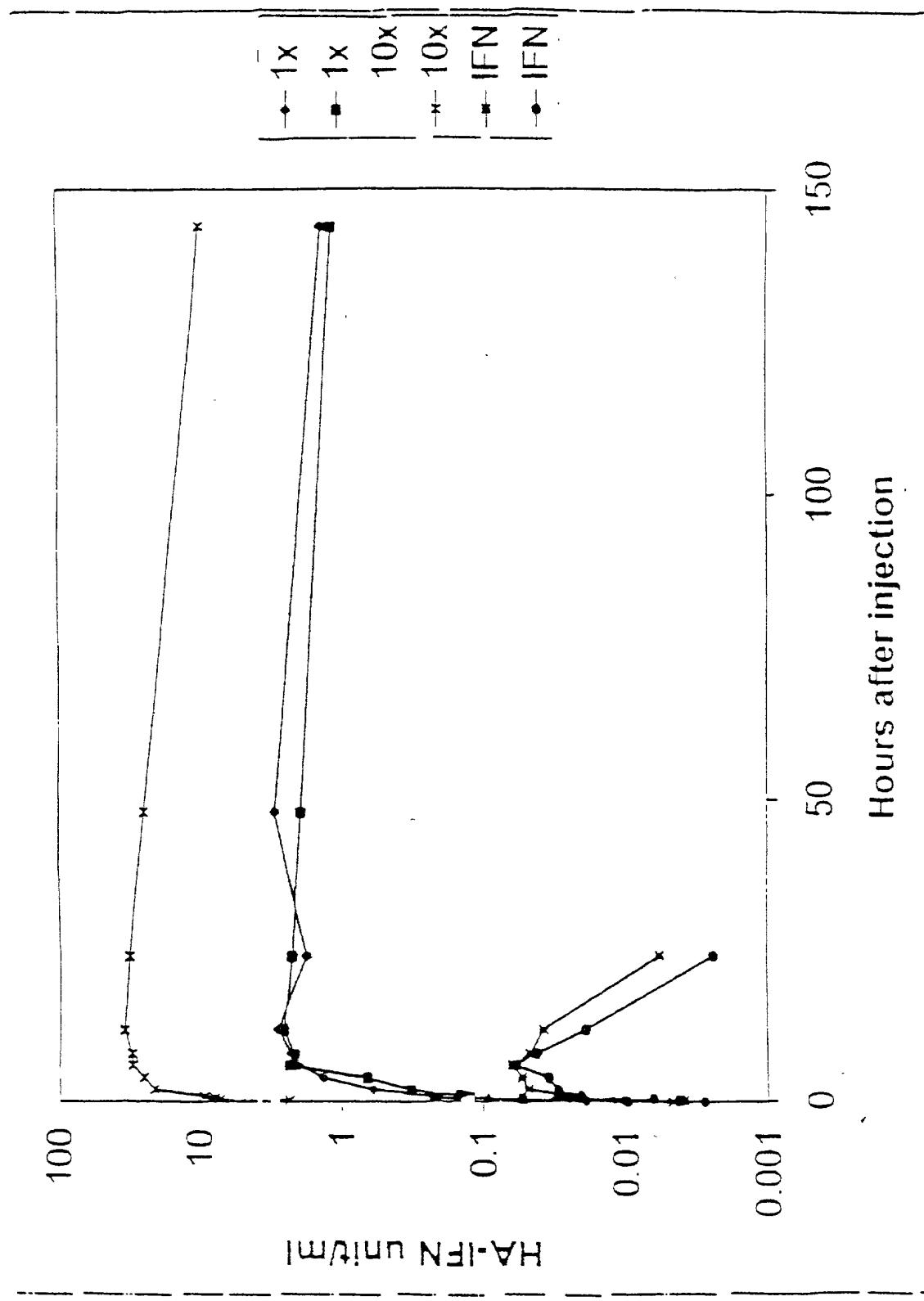
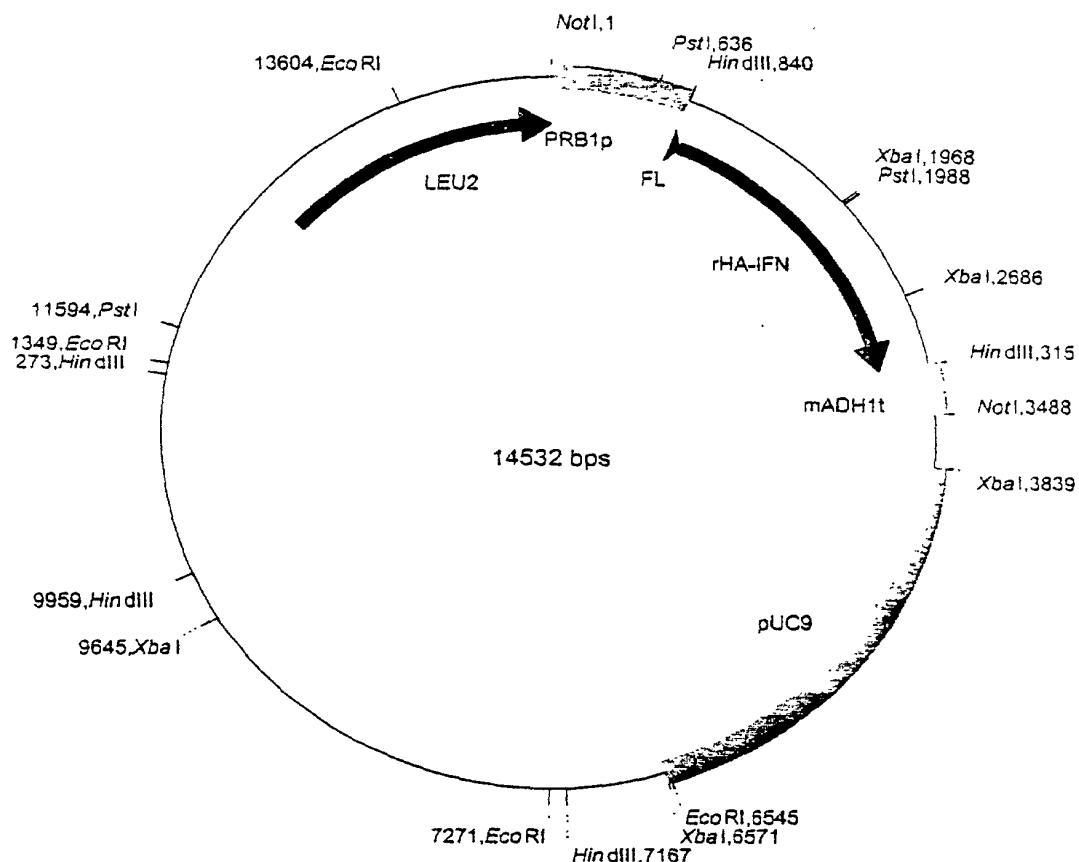


Figure 7





**Figure 8.** The HA-IFN $\alpha$  expression cassette in pSAC35. The expression cassette comprises  
*PRB1* promoter, from *S. cerevisiae*.  
 Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF $\alpha$ -1 leader.  
 HA-IFN $\alpha$  coding sequence with a double stop codon (TAATAA)  
*ADH1* terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III/BamHI* fragment generally used.

**Figure 8**

## Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

**Figure 9**

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH

IV

151 APELLFFAKR YKAATTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

**b. Insertion (or replacement) of Randomised sequence into Loop IV.**

(X)<sub>n</sub>

↓

IV

151 APELLFFAKR YKAATTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC  
 HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH HHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

**Figure 10**

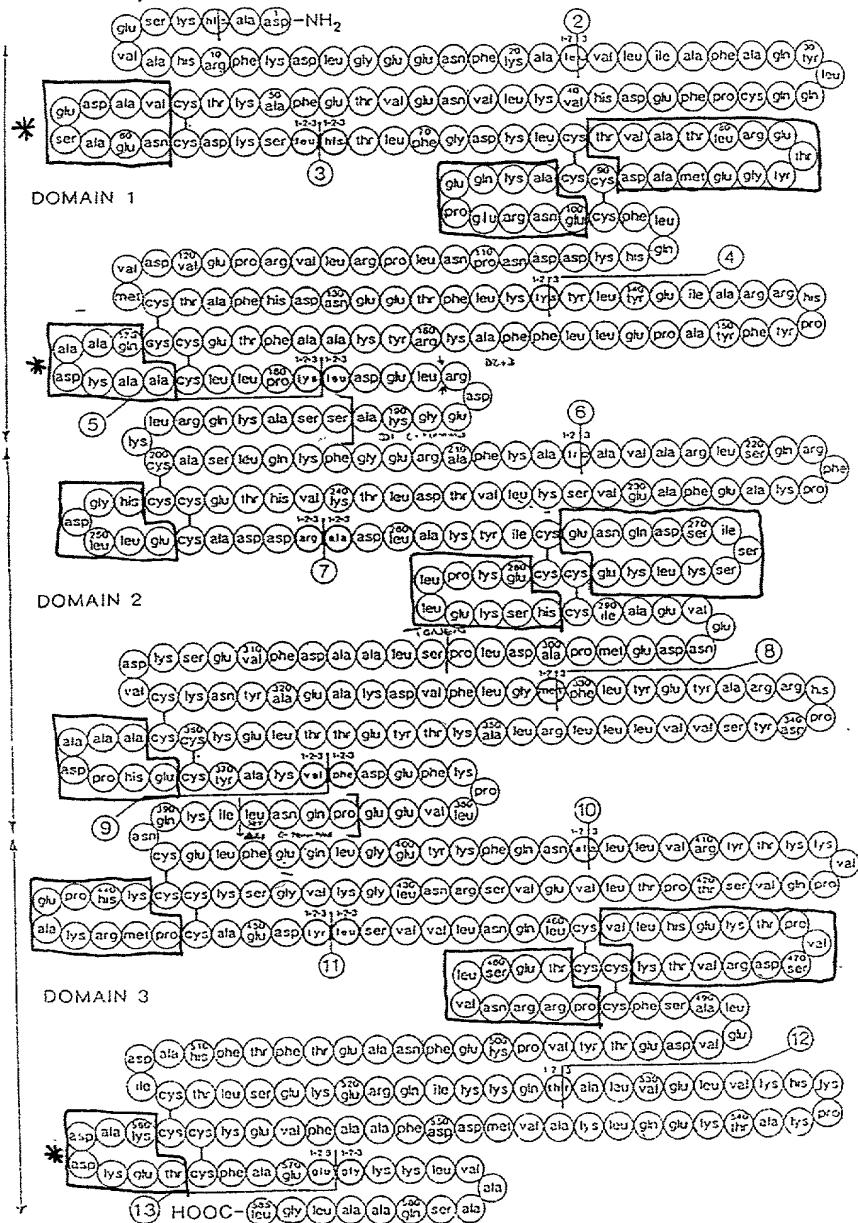
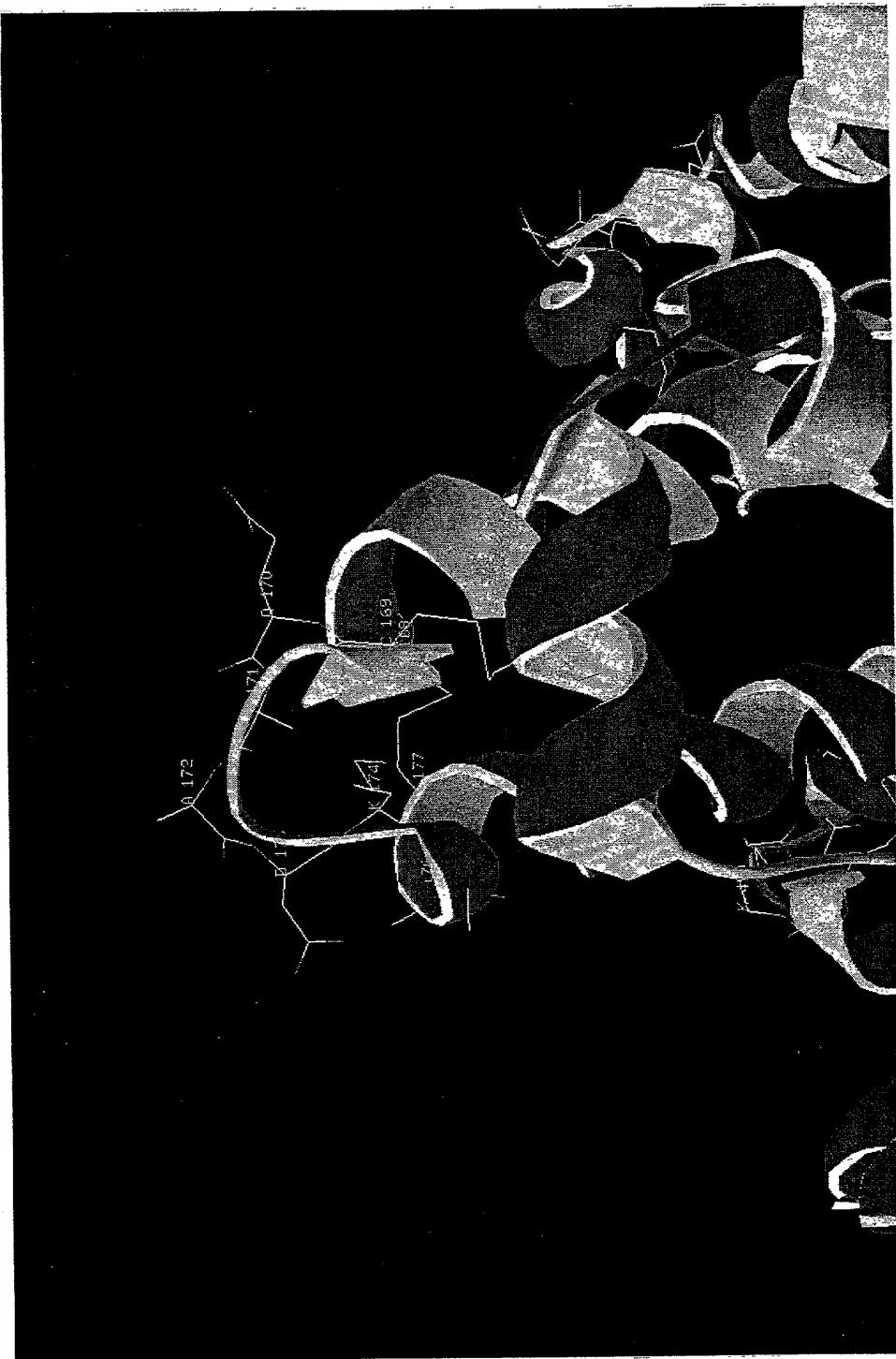


Figure 11



Disulfide bonds shown in yellow

**Figure 12: Loop IV Gln170-Ala176**

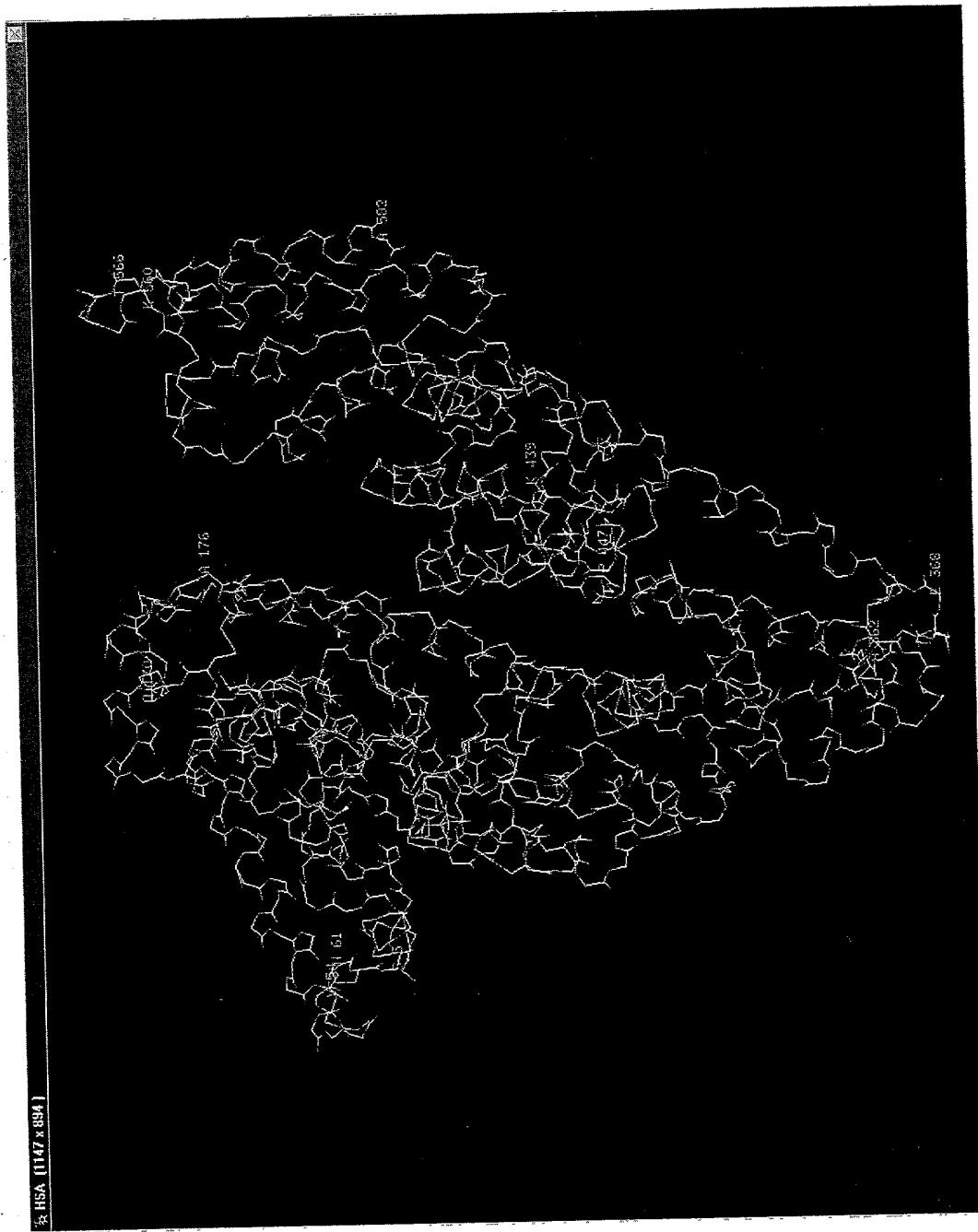
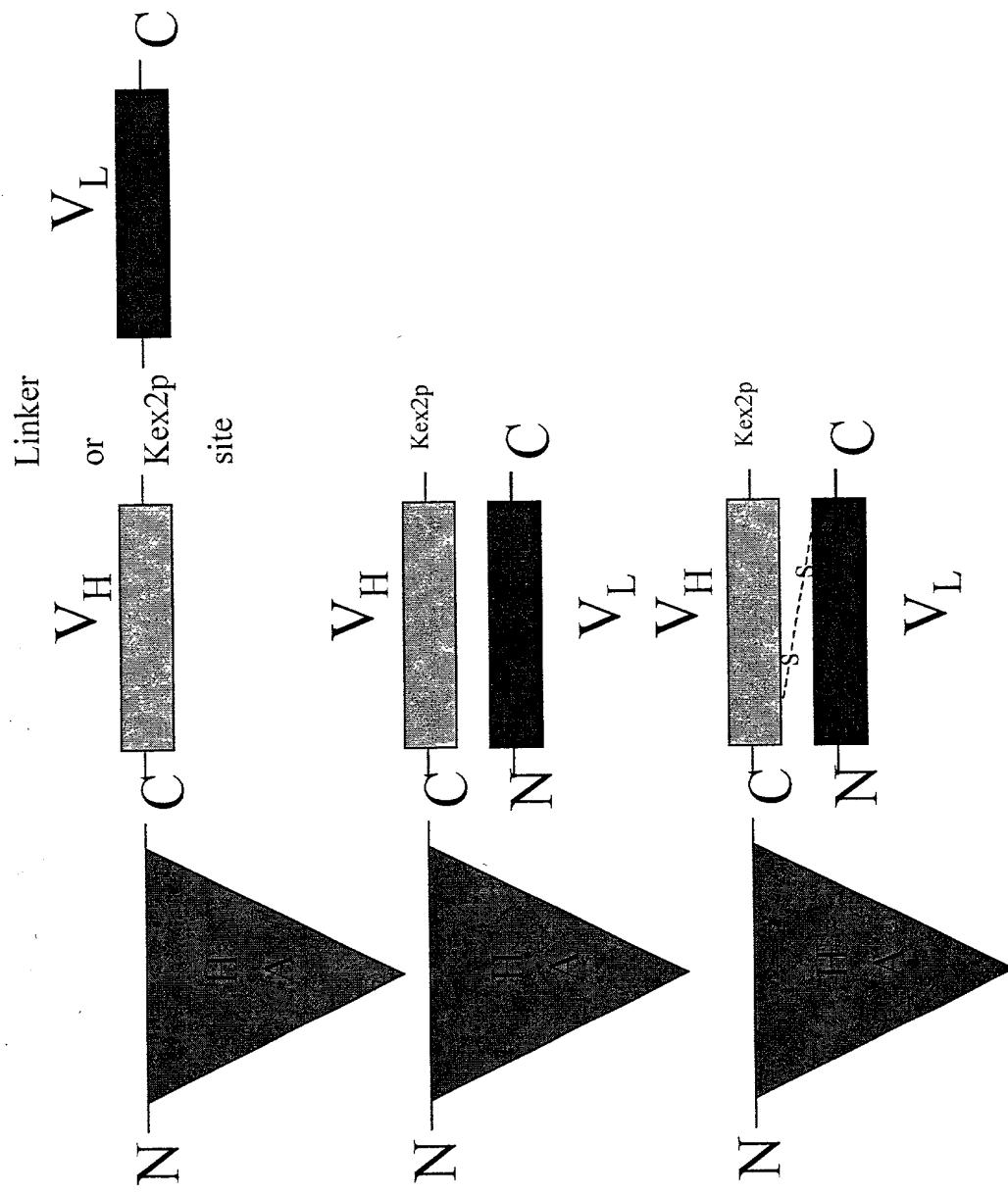


Figure 13: Tertiary Structure of HA



**Figure 14: Schematic Diagram of Possible ScFv Fusions  
(Example is of a C-terminal fusion to HA)**

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1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60  
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120  
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180  
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240  
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300  
81 R E T Y G E M A D C C A K Q E P H R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360  
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420  
121 D V M C T A F H D N E E T F L K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480  
141 E I A R R P Y F V A P E L L F A K R 160

Figure 15A

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481	TAT	AAA	GCT	GCT	TTT	ACA	GAA	TGT	TGC	CAA	GCT	GAT	AAA	GCT	GCC	TGC	CTG	TTG	CCA	540	
161	Y	K	A	A	F	T	E	C	C	Q	A	D	K	A	A	C	L	L	P	180	
541	AAG	CTC	GAT	GAA	CTT	CGG	GAT	GAA	GGG	AAG	GCT	TCG	TCT	GCC	AAA	CAG	AGA	CTC	AAA	TGT	600
181	K	L	D	E	L	R	D	E	G	K	A	S	S	A	K	Q	R	L	K	C	200
601	GCC	AGT	CTC	CAA	AAA	TTT	GGA	GAA	AGA	GCT	TTC	AAA	GCA	TGG	GCA	GTG	GCT	CGC	CTG	AGC	660
201	A	S	L	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	220
661	CAG	AGA	TTT	CCC	AAA	GCT	GAG	TTT	GCA	GAA	GTT	TCC	AAG	TTA	GTG	ACA	GAT	CTT	ACC	AAA	720
221	Q	R	F	P	K	A	E	F	A	E	V	S	K	L	V	T	D	L	T	K	240
721	GTC	CAC	ACG	GAA	TGC	TGC	CAT	GGA	GAT	CTG	CTT	GAA	TGT	GCT	GAT	GAC	AGG	GCG	GAC	CTT	780
241	V	H	T	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L	260
781	GCC	AAG	TAT	ATC	TGT	GAA	AAT	CAG	GAT	TCG	ATC	TCC	AGT	AAA	CTG	AAG	GAA	TGC	TGT	GAA	840
261	A	K	Y	I	C	E	N	Q	D	S	I	S	S	K	L	K	E	C	C	E	280
841	AAA	CCT	CTG	TTG	GAA	AAA	TCC	CAC	TGC	ATT	GCC	GAA	AAT	GAT	GAG	ATG	CCT	GCT	GCT	900	
281	K	P	L	L	E	K	S	H	C	I	A	E	V	E	N	D	E	M	P	A	300
901	GAC	TTG	CCT	TCA	TTA	GCT	GCT	GAT	TTT	GTT	GAA	AGT	AAG	GAT	GTT	TGC	AAA	AAC	TAT	GCT	960
301	D	L	P	S	L	A	A	D	F	V	E	S	K	D	V	C	K	N	Y	A	320

Figure 15B

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961	GAG	GCA	AAG	GAT	GTC	TTC	CTG	GCC	ATG	TTG	TTG	TAT	GAA	TAT	GCA	AGA	AGG	CAT	CCT	GAT	1020	
321	E	A	K	D	V	F	L	G	M	F	L	Y	E	Y	A	R	R	R	H	P	D	340
1021	TAC	TCT	GTC	GTG	CTG	CTG	AGA	CTT	GCC	AAG	ACA	TAT	GAA	ACC	ACT	CTA	GAG	AAG	TGC	1080		
341	Y	S	V	V	L	L	R	L	A	K	T	Y	E	T	T	L	E	K	C	360		
1081	TGT	GCC	GCT	GCA	GAT	CCT	CAT	GAA	TGC	TAT	GCC	AAA	GTC	TTG	TTC	GAT	GAA	TTT	AAA	CCT	CTT	1140
361	C	A	A	D	P	H	E	C	Y	A	K	V	F	D	E	F	K	P	L	380		
1141	GTG	GAA	GAG	CCT	CAG	AAT	TTA	ATC	AAA	CAA	AAC	TGT	GAG	CTT	TTT	GAG	CAG	CTT	GGA	GAG	1200	
381	V	E	E	P	Q	N	L	I	K	Q	N	C	E	L	F	E	Q	L	G	E	400	
1201	TAC	AAA	TTC	CAG	AAT	GCG	CTA	TTA	GTT	CGT	TAC	ACC	AAG	AAA	GTA	CCC	CAA	GTG	TCA	ACT	1260	
401	Y	K	F	Q	N	A	L	L	V	R	Y	T	K	K	V	P	Q	V	S	T	420	
1261	CCA	ACT	CTT	GTA	GAG	GTC	TCA	AGA	AAC	CTA	GGA	AAA	GTC	GCG	AGC	AAA	TGT	TGT	AAA	CAT	1320	
421	P	T	L	V	E	V	S	R	N	L	G	K	V	G	S	K	C	C	K	H	440	
1321	CCT	GAA	GCA	AAA	AGA	ATG	CCC	TGT	GCA	GAA	GAC	TAT	CTA	TCC	GTG	GTC	CTG	AAC	CAG	TTA	1380	
441	P	E	A	K	R	M	P	C	A	E	D	Y	L	S	V	V	L	N	Q	L	460	
1381	TGT	GTG	TTG	CAT	GAG	AAA	ACG	CCA	GTA	AGT	GAC	AGA	GTC	ACA	AAA	TGC	TGC	ACA	GAG	TCC	1440	
461	C	V	L	H	E	K	T	P	V	S	D	R	V	T	K	C	C	T	E	S	480	

Figure 15C

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1441	T	TG	G	TG	A	A	C	AGG	CGA	CCA	TGC	TTT	TCA	GCT	CTG	GAA	GTC	GAT	GAA	ACA	TAC	GTT	CCC	AAA	1500	
481	L	V	N	R	R	P	C	F	S	A	L	E	V	D	E	T	Y	V	P	K	500					
1501	G	A	G	T	TT	A	T	G	C	T	G	A	C	T	T	G	A	G	A	1560						
501	E	F	N	A	E	T	F	T	F	H	A	D	I	C	T	L	S	E	K	E	520					
1561	A	G	C	AA	ATC	A	G	AA	CAA	ACT	GCA	CTT	GTT	GAG	CTT	GTC	AAA	CAC	AAG	CCC	AAG	GCA	ACA	1620		
521	R	Q	I	K	K	Q	T	A	L	V	E	L	V	K	H	K	P	K	A	T	540					
1621	A	AA	G	G	C	AA	CTG	AAA	GCT	GTT	ATG	GAT	GAT	TTC	GCA	GCT	TTT	GTA	GAG	AAG	TGC	TGC	AAG	1680		
541	K	E	Q	L	K	A	V	M	D	D	F	A	A	F	V	E	K	C	C	K	560					
1681	G	C	T	G	A	G	G	AC	T	G	T	T	G	G	G	G	G	AAA	AAA	CTT	GTT	GCT	GCA	AGT	CAA	1740
561	A	D	D	K	E	T	C	F	A	E	E	G	K	K	L	V	A	A	S	Q	580					
1741	G	C	T	T	A	GGC	TTA	TAA	CAT	CTA	CAT	TTA	AAA	GCA	TCT	CAG	1782									
581	A	A	L	G	L	*															585					

Figure 15D